National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2670a

Toxic Elements in Urine (Freeze-Dried)

This Standard Reference Material (SRM) is primarily intended for use in evaluating the accuracy of clinical methods and for the calibration of apparatus used to determine the concentration of toxic metals and other elements in human urine or similar matrices. It can also be used to validate working or secondary reference materials. A unit of SRM 2670a consists of four bottles of freeze-dried urine, two bottles each at the low and high levels. Before use, the urine in each bottle is to be reconstituted with 20.00 mL of high purity deionized water (see *Instructions for Use*). The low level urine was prepared from human urine that was lyophilized after pooling and centrifugation. The high level urine was prepared by spiking an aliquot of the pooled and homogenized low-level urine with selected metals, followed by lyophilization. Due to the centrifugation (which improved sample homogeneity), neither level represents a fresh urine pool from a normal human population.

The certified, reference, and information concentration values apply only to properly reconstituted urine at room temperature (20 °C to 25 °C, see *Instructions for Use*).

Expiration of Certification: The certification of this SRM is valid within the measurement uncertainties specified until **31 December 2012**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see *Instructions for Use*). However, the certification will be nullified if the SRM is contaminated or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

Analytical measurements on this SRM were performed by C.M. Beck II, T.A. Butler, W.R. Kelly, S.E. Long, E.A. Mackey, J.L. Mann, M.S. Rearick, R.D. Vocke, Jr., L.J. Wood and L.L. Yu and by Ö. Ertaş, a Research Associate at NIST in the NIST Analytical Chemistry Division. R.L. Jones and G. Shakirova at the Centers for Disease Control and Prevention (CDC), Atlanta, GA and D.E. Nixon at the Mayo Clinic, Rochester, MN performed additional analytical measurements.

The CDC provided partial financial support for the development of this SRM under the direction of project officers E.W. Gunter, D.C. Paschal, and R.L. Jones of the National Center for Environmental Health, Division of Laboratory Sciences, Atlanta, GA.

The lyophilization and bottling operations were carried out by Bio-Rad Laboratories, Hercules, CA.

C. Hagwood of the NIST Statistical Engineering Division provided consultation on the evaluation of the data.

The overall direction and coordination of the analyses were under the chairmanship of R.D. Vocke, Jr. and G.C. Turk of the NIST Analytical Chemistry Division.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by B.S. MacDonald of the NIST Measurement Services Division.

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Certificate Issue Date: 11 August 2003

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NOTICE AND WARNINGS TO USERS: SRM 2670a IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. THIS IS A HUMAN SOURCE MATERIAL AND SHOULD BE TREATED AS A BIOHAZARDOUS SUBSTANCE CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. THE RECONSTITUTED URINE SHOULD BE HANDLED WITH PRECAUTIONS SUITABLE FOR FRESH URINE.

INSTRUCTIONS FOR USE: In order for the Certified concentrations to be valid, this SRM must be reconstituted as follows. Remove the bottle from the refrigerator, and allow it to equilibrate at room temperature before reconstitution. Carefully remove the metal seal. Take extra care in removing the rubber stopper, as some of the lyophilized urine may adhere to it. Using a Type I, Class A calibrated volumetric transfer pipette or other dispenser of known accuracy, add 20.00 mL of CAP/NCCLS Type I water [1] or equivalent to each bottle. After replacing the stopper, the bottle should be allowed to stand at room temperature with occasional swirling for 30 minutes to ensure complete dissolution. **DO NOT SHAKE**. Vigorous shaking causes foaming, which may lead to an inhomogeneous distribution of the analytes within the bottle. Allow 2 h for reconstitution. After reconstitution, the contents should be used immediately or stored between 2 °C and 8 °C until ready for use, preferably within 12 h. Density measurements made on the reconstituted material with a micro-pycnometer gave values of 1.0024 ± 0.0003 g/mL for both levels of SRM 2670a.

Stability and Storage: The urine comprising SRM 2670a is lyophilized (freeze-dried) material and should be stored in a refrigerator at a temperature between 2 °C and 8 °C until ready for use. It should not be frozen or exposed to sunlight or ultraviolet radiation. After reconstitution, the contents should be used immediately or stored between 2 °C and 8 °C until ready for use, preferably within 12 h. Some of the elements, most notably Hg, are volatile and are progressively lost after reconstitution. Freezing of the reconstituted material is not recommended.

Certified Values and Uncertainties: Certified concentration values for 10 elements in the low level and 14 elements in the high level of SRM 2670a are listed in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST. All values are reported on a mass/volume basis [2] (see *Instructions for Use*) and are based on measurements using the entire lyophilized sample mass.

Table 1. Certified Concentration Values

	Low Level				High Level			
Element	**Quantity**	**Units**	**Uncertainty**	***k***	**Quantity**	**Units**	**Uncertainty**	***k***
Antimony	0.971	µg/L	± 0.033	2.45	0.824	µg/L	± 0.070	2.57
Cadmium	0.0591	µg/L	± 0.0034	2.36	4.862	µg/L	± 0.084	2.36
Cesium	1.075	µg/L	± 0.031	2.57	1.085	µg/L	± 0.052	2.31
Cobalt	0.166	µg/L	± 0.040	2.09	51.2	µg/L	± 3.2	2.23
Iodine[a]	88.2	µg/L	± 1.1	2.00	88.2	µg/L	± 1.1	2.00
Lead	0.49	µg/L	± 0.16	2.57	233.2	µg/L	± 9.4	2.57
Mercury	0.0663	µg/L	± 0.0058	2.57	95.1	µg/L	± 0.98	2.00
Manganese	---	---	---	---	99	µg/L	± 12	2.78
Molybdenum	---	---	---	---	114.1	µg/L	± 4.8	2.01
Platinum	---	---	---	---	51.5	µg/L	± 6.6	2.00
Selenium	---	---	---	---	229.5	µg/L	± 8.3	2.57
Thallium	0.0162	µg/L	± 0.0045	3.18	5.417	µg/L	± 0.064	2.36
Thorium	0.0053	µg/L	± 0.0014	2.57	0.01606	µg/L	± 0.00077	2.45
Uranium	0.1020	µg/L	± 0.0023	2.57	4.997	µg/L	± 0.071	4.30

[a] *Iodine concentrations, as measured, are for Iodide*

The certified values for cadmium, iodine, lead, mercury, thallium, thorium and uranium are the means of results obtained by NIST using isotope dilution-inductively coupled plasma mass spectrometry (ID-ICPMS). The expanded uncertainties are calculated as prediction intervals where $U = ku_c$. The uncertainty component u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO Guide [3]. The coverage factor, k, is determined from the Student's t-distribution for the appropriate

degrees of freedom to yield 95 % confidence. The certified values and uncertainties for the remaining elements are derived from the results of at least one analysis performed at NIST and independent results from one or more methods provided by the CDC (Atlanta, GA) and/or the Mayo Clinic, (Rochester, Minnesota) using the approach described by Levenson, *et al.* [4] for combining results from multiple methods. Multiple method results from the laboratories outside NIST were first combined to give a single value and uncertainty before being combined with the NIST results. The certified value is an unweighted mean of the results from NIST and these laboratories. The uncertainty listed with each value is an expanded uncertainty about the mean, $U = ku_c$, with a coverage factor k determined from the Student's t-distribution for the appropriate degrees of freedom to yield 95 % confidence. Each u_c is calculated by combining a between-method variance [4] with a pooled, within-method variance [3]. Analytical methods are listed in Appendix A.

Reference Values and Uncertainties: Reference concentration values for 7 elements at the low level and 8 elements at the high level are given in Table 2. Reference values are non-certified values that are the best estimate of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may not include all sources of uncertainty.

Table 2. Reference Concentration Values

	Low Level			High Level		
Element	**Quantity**	**Units**	**Uncertainty**	**Quantity**	**Units**	**Uncertainty**
Calcium	29	mg/L	± 2	30	mg/L	± 2
Magnesium	21.0	mg/L	± 0.2	21.2	mg/L	± 0.2
Potassium	410	mg/L	± 10	415	mg/L	± 10
Sodium	856	mg/L	± 15	942	mg/L	± 20
Arsenic	---	---	---	220	µg/L	± 10
Copper	---	---	---	110	µg/L	± 4
Manganese	2.6	µg/L	± 0.7	---	---	---
Selenium	8	µg/L	± 3	---	---	---
Tin	---	---	---	89	µg/L	± 7
Zinc	130	µg/L	± 30	410	µg/L	± 30

The reference concentration values are based either on the results of a single NIST method or on the results of a single NIST method and one or more outside laboratories methods. Reference values and uncertainties were derived from multiple results in the same manner as was done for the certified values and uncertainties. Zinc, an important analyte in urine, was not certified in SRM 2670a due to possible contamination from the stopper used in the packaging. Analytical methods are listed in Appendix A.

Information Values: Information concentration values are provided in Table 3. An information value is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess the uncertainty associated with the value.

Table 3. Information Concentration Values

	Low Level		High Level	
Element	**Quantity**	**Units**	**Quantity**	**Units**
Aluminum	4	µg/L	100	µg/L
Arsenic	3	µg/L	---	---
Barium	2	µg/L	2	µg/L
Beryllium	---	µg/L	5	µg/L
Chromium	2	µg/L	20	µg/L
Copper	5	µg/L	---	---
Molybdenum	17	µg/L	---	---
Nickel	2	µg/L	100	µg/L
Tin	<1	µg/L	---	---
Tungsten	<1	µg/L	<1	µg/L
Vanadium	<1	µg/L	30	µg/L

REFERENCES

[1] *Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline – Third Edition*, NCCLS document C3-A3, NCCLS, Wayne, Pennsylvania (1997).

[2] Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811, 1995 Ed.; U.S. Government Printing Office: Washington, DC (1995).

[3] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/.

[4] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H.K.; Vangel, M.G.; Yen, J.H.; Zhang, N.F.; *An Approach to Combining Results from Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571-579 (2000).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.

Appendix A. Methods Used in Analyte Determinations

Method	Analytes Determined
Cold Vapor Isotope Dilution Inductively Coupled Plasma Mass Spectrometry at NIST	Hg
Isotope Dilution Inductively Coupled Plasma Mass Spectrometry at NIST	Cd, I, Pb, Th, Tl, U
Standard Additions Inductively Coupled Plasma Mass Spectrometry at NIST	Mn, Al, V
Inductively Coupled Plasma Mass Spectrometry at NIST (High Level only)	As, Co, Cu, Mn, Mo, Pt, Se, Sn
Instrumental Neutron Activation Analysis at NIST	Co, Cs, Sb, Se, Zn
Inductively Coupled Plasma Atomic Emission Spectrometry at NIST	Ca, K, Mg
Flame Emission Spectrometry at NIST	Na
Inductively Coupled Plasma Mass Spectrometry at the CDC, Atlanta, GA	Be, Ba, Cd, Co, Cs, Mo, Pb, Pt, Sb, Tl, U, W
Inductively Coupled Plasma Mass Spectrometry at the Mayo Clinic, Rochester, MN	Al, As, Cd, Co, Cr, Cu, Hg, I, Mn, Mo, Ni, Pb, Pt, Sb, Se, Sn, Tl, V, Zn
Electrothermal Vaporization Atomic Absorption Spectrometry at the Mayo Clinic, Rochester, MN	Al, Cr, Mn